Reference: *Biol. Bull.* **160:** 366–375. (June, 1981)

PROTEIN SYNTHESIS IN THE POLAR LOBE AND LOBELESS EGG OF *ILYANASSA OBSOLETA*

J. R. COLLIER

Biology Department, Brooklyn College, Brooklyn, N. Y. 11210

Abstract

The relative rates of protein synthesis in the lobeless egg and polar lobe of *Ilyanassa obsoleta* were measured and found to be the same. Two-dimensional electrophoresis of ^{35}S-methionine *in vivo* labeled polypeptides extracted from the *Ilyanassa* egg and its isolates resolved the same set of polypeptides from the egg, lobeless egg, and isolated polar lobe. For those peptides detected it was concluded that (1) the lobeless egg and the polar lobe contain the same set of mRNAs, (2) these mRNAs are not differentially translated in either of these isolates, (3) the polar lobe does not regulate the synthesis of these proteins, either quantitatively or qualitatively, during early cleavage, and (4) the peptides synthesized in the isolated polar lobe were translated from mRNAs produced during oogenesis.

It was shown by one-dimensional electrophoresis of ^{35}S-methionine-labeled basic peptides that the egg, lobeless egg, and isolated polar lobe synthesized, at approximately the same rate, two variants of H1 histone, histones H2A, H2B, H3, and H4, and high mobility group peptides 14 and 17. Because these peptides were synthesized in the isolated polar lobe it was concluded that they were translated from maternal mRNAs.

Introduction

The significance of the role played by egg cytoplasm in determination and differentiation was established in the early embryological literature (Wilson, 1928). The isolation of the polar lobe and the separation of blastomeres by Crampton (1896) and Clement (1952, 1956) of the egg of *Ilyanassa obsoleta*, the common mud snail of the North American Atlantic coast, demonstrated the importance of the localization of specific kinds of cytoplasm within this egg and the selective distribution of egg cytoplasm to the early blastomeres.

Clement and Tyler (1967) demonstrated that the isolated polar lobe, a vegetal merogone lacking a nucleus, can synthesize proteins. The influence of removing the polar lobe on the embryo's synthesis of individual polypeptides has been investigated recently (Newrock and Brandhorst, 1979; Collier and McCarthy, 1979; McCarthy and Collier, 1980).

The present investigation of protein synthesis during early cleavage was prompted by the question of whether the expression of structural genes is involved in the early determinative events of this spiralian egg. Relative rates of protein synthesis by different regions of the egg were measured by determining the incorporation of radioactive amino acids into acid-precipitable proteins and by two-dimensional electrophoresis of *in vivo* labeled peptides. The contribution of mito-

Received 29 January 1981; accepted 6 March 1981.

Abbreviations: HMG, high mobility group; NP-40, Tergito, type NP-40; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid; EDTA, ethylenediaminetetraacetic acid.

chondria to protein synthesis was assessed, and the synthesis of histones and high mobility group peptides was determined in the *Ilyanassa* egg and the polar lobe.

Materials and Methods

Maintenance of snails and rearing of embryos

Mud snails collected at Plumb Beach in Brooklyn, New York, were kept in a 20-gal tank of recirculating sea water. Fed one clam on alternate days, they deposited a few hundred fresh egg capsules daily. Eggs and embryos were reared in filtered sea water from the Supply Department of the Marine Biological Laboratory, Woods Hole, Massachusetts, with 200 μg/ml each of streptomycin and pencillin added. Polar lobes were isolated in sea water low in calcium and magnesium, as previously described (Collier, 1975). Embryos were reared at 19°C and staged by time of development from the two-cell stage.

All groups of embryos designated for direct comparison, *e.g.* normal and lobeless embryos, were obtained from a common pool of eggs.

Labeling of proteins

Proteins were labeled *in vivo* by rearing embryos for 4 h in 120 μCi/ml of L(^{35}S)-methionine (New England Nuclear, 800–1100 Ci mmol^{-1}). Radioactive methionine was dried *in vacuo* in a small glass vial to which was added 100–200 embryos in 0.3 ml of sea water containing penicillin and streptomycin. This vial was continuously rotated in a Rollerdrum (New Brunswick Scientific Co.) for 4 h at room temperature (24°C).

For the experiments with cycloheximide, embryos were labeled by incubation in 200–500 μCi/ml of ^{3}H-leucine (53.8 Ci mmol^{-1}, New England Nuclear) by the procedure described above.

Measurement of uptake and incorporation of amino acids

Labeled embryos or polar lobes were thoroughly rinsed with sea water, counted, placed in 0.2 ml of 2% SDS (sodium dodecyl sulfate) and heated at 100°C for 3 min. One portion of the SDS solubilized protein was counted in Bray's (1960) solution to determine the total radioactivity in the embryos. Another portion was precipitated onto a Millipore filter from cold 10% TCA (trichloroacetic acid), washed with 5% TCA, and counted in a scintillation spectrometer by dissolving the filter in Bray's solution.

Sample preparation for two-dimensional electrophoresis

Embryos labeled with ^{35}S-methionine were washed twice in calcium-magnesium-free sea water; suspended in 0.05 ml of this sea water; mixed vigorously for 30 s in an equal volume of homogenizing solution (0.01 *M* EDTA (ethylenediaminetetraacetic acid), 0.1 *M* methionine, 1.0% SDS, and 10% mercaptoethanol); and then heated to 100°C for 3 min. This solubilized homogenate was cooled, frozen, and lyophilized to dryness. The lyophilized homogenate was dissolved in 0.1 ml of a sample buffer containing 8.5 *M* urea (Schwarz-Mann Ultrapure), 2.0% NP-40 (Tergito, type NP-40; Sigma Chemical Co.), 0.1 *M* dithiothreitol, 0.05 *M* EDTA, 1.2% pH 3–10 ampholyte, and 1.6% pH 5–7 ampholyte (all ampholytes

were obtained from BioRad Laboratories). The sample was then stored at −70°C.

Electrophoresis

Two-dimensional electrophoresis was carried out as described by O'Farrell (1975) with the following modifications: the isoelectric focusing gels contained 8.5 *M* urea and 2.8% ampholytes (1.2% pH 3–10 and 1.6% pH 5–7 Biolyte obtained from BioRad Laboratories). The gels were polymerized in 10.5 cm × 0.3 cm Lucite tubes that were held at 25°C during electrophoresis for 8400 volt-hours.

Second dimension electrophoresis of the first dimension gels was on discontinuous SDS slab gels, 16.0% acrylamide separating gels, and 5.4% acrylamide stacking gels, as described by Laemmli (1970), except that the gels contained 0.15% instead of 0.8% bisacrylamide. The dried gels were prepared for fluorography as described by Garrells (1979) and exposed to Kodak XR-5 X-ray film.

The volume of all samples used for electrophoresis was adjusted so that each sample designated for comparison contained the same mass of protein. Variation in radioactivity of the samples was compensated for by the exposure time of the autoradiogram.

Electrophoresis of histones

Embryos labeled with ^{35}S-methionine as described above were washed in several changes of sea water and solubilized by heating at 100°C for 3 min in 0.0625 *M* Tris, pH 6.8, containing 2% SDS and 50 m*M* dithiothreitol. Bromophenol Blue tracking dye was added to the sample, which was then frozen until used. The frozen samples were thawed and loaded onto a discontinuous acrylamide slab gel as formulated by Laemmli (1970), but modified to contain 0.15% instead of 0.8% bisacrylamide. The separating gel contained 16% acrylamide. Electrophoresis was continued until the tracking dye moved off of the gel.

The position of the histones on the autoradiograph was established by co-electrophoresis of ^{35}S-methionine labeled basic proteins with histones extracted from isolated nuclei of *Ilyanassa* embryos. The slab gel was stained with Coomassie Blue and then dried. The dried gel and a sheet of X-ray film were held in registry while both were notched several times along two sides. The gel and film were pinned together and the film was exposed to the gel for the required time. After the film had been developed this marking system provided a precise matching of the autograph with the stained gel, and obviated mismatching between the position of the stained histones (Fig. 2D) and radioactive bands on the autoradiograph from changes in the gel dimension during processing.

Results

Mitochondrial protein synthesis

Generally, mitochondria are not expected to make a major contribution to polypeptide synthesis. However, the relatively low level of protein synthesis during early cleavage and the small amount of cytoplasm in the polar lobe require an assessment of mitochondrial protein synthesis. Accordingly, the effect of cycloheximide on protein synthesis during early cleavage was investigated.

Four-cell embryos were pulsed for 1 h with 200 μCi/ml of ^{3}H-leucine, washed with sea water, and transferred to varying concentrations of cycloheximide for the

TABLE I

Cycloheximide inhibition of protein synthesis during early cleavage. Leucine incorporation into protein as CPM per embryo

	Cycloheximide concentration micrograms per ml			
Time, min	0	100	200	400
60	230	257	373	327
	398	206	361	318
	441	155	206	327
Mean	356	206	313	313
	***†	***	NS	NS
120	581	570	418	393
	515	504	375	320
	548	561	333	261
Mean	548	545	375	324

† ***, $P < 0.01\%$; NS, $P > 0.05\%$ for significance of differences between means for treatment at 60 and 120 min.

times indicated in Table I. After 1 h 400 μg/ml of cycloheximide, a differential inhibitor of non-mitochondrial protein synthesis (Schatz and Mason, 1974), inhibited 96.5% of the incorporation of leucine into protein. Thus, little, if any, intramitochondrial protein synthesis occurred during early cleavage.

Relative rates of polypeptide synthesis in the egg, lobeless egg, and polar lobe

Table II shows the results of an experiment in which eggs were pre-loaded with ^{35}S-methionine for 1.5 h before the trefoil stage, removed from precursor, and delobed immediately. The total uptake of ^{35}S-methionine and its incorporation into protein was then determined for the egg, lobeless egg, and polar lobe. This experiment avoids the problem of differential uptake of precursor by any of the isolates, and measures the precursor's compartmentation into the lobeless egg and polar lobe. Table II, Part A shows that 77.1% of the total ^{35}S-methionine was partitioned into the lobeless egg and 16.9% into the polar lobe; similarly, Table II, Part B shows that these parts of the egg account for 76.8% and 12.9%, respectively, of the ^{35}S-methionine incorporated into protein. These distributions are not significantly different ($F(1, 4) = 1.58$, $P > 0.05$) from the 84.2% of the volume of non-yolk cytoplasm in the lobeless egg and 16.5% in the polar lobe (Collier, 1957). From this distribution of radioactive amino acids, I infer that the egg's amino acid pool, a quantity not directly measurable in a small number of eggs or isolates, is distributed into the lobeless egg and the polar lobe in proportion to their cytoplasmic volume. Accordingly, the ^{35}S-methionine incorporation into protein, insofar as it depends on the size of the amino acid pool, should be proportional to the cytoplasmic volume of the lobeless egg and the polar lobe. The data on the incorporation of radioactivity into protein per unit of cytoplasm (Table II, Part C) shows that this is the case. The measured values for this incorporation are not significantly different ($F(2, 6) = 2.23$, $P > 0.05$) for the egg, lobeless egg, or polar lobe. Thus, the egg and its isolates are synthesizing polypeptides at the same relative rates.

Electrophoresis of labeled polypeptides

Whether the average relative rate of protein synthesis, as measured by the incorporation of ^{35}S-methionine into acid-precipitable proteins, accurately reflected

TABLE II

Uptake and incorporation of ^{35}S-methionine

	Radioactivity and per cent distribution of radioactivity*				
	Egg	Lobeless egg		Polar lobe	
	CPM	CPM	%	CPM	%
A. Total radioactivity:	159,003	118,677	74.6	31,240	19.6
	172,277	130,461	75.7	31,916	18.5
	125,532	101,730	81.0	15,808	12.6
Mean %			77.1		16.9
B. Acid precipitable CPM	33,414	27,251	81.6	5,424	16.2
	40,956	29,012	70.8	5,356	13.1
	26,073	20,341	78.0	2,436	9.4
Mean %			76.8		12.9
C. CPM $\times 10^7$ incorporated per mm^3 cytoplasm:	2.5	2.4		2.5	
	3.1	2.6		2.4	
	2.0	1.8		1.1	
Mean	2.5	2.3		2.0	

* Significance of differences among groups was determined by an analysis of variance; F-distribution, degrees of freedom and *P* values are in the text.

the rate of synthesis of individual peptides was determined by separating polypeptides by two-dimensional electrophoresis on acrylamide gels. For these studies, ^{35}S-methionine was used because of its high specific activity and the high energy of its beta emission. These features made possible the use of a small number of embryos and relatively short exposure times for the autoradiographs. In Figure 1 are autofluorographs of two-dimensional gels of peptides extracted from eggs, lobeless eggs, and isolated polar lobes labeled *in vivo* from the two- to the twelve-cell stage with ^{35}S-methionine. These gels were loaded with the same amount of total cytoplasmic protein, *i.e.* total protein minus yolk protein. Differences in radioactivity loaded onto each gel were compensated for by the length of exposure of the autoradiographs. Comparing the position, size, and density of spots among these autofluorographs (Fig. 1) shows that the egg and its isolates synthesized the same set of peptides at the same relative rate of accumulation.

Synthesis of histones and high mobility group peptides

The two-dimensional electrophoresis of peptides described above did not resolve basic peptides. However, histones labeled *in vivo* with ^{35}S-methionine in normal eggs, lobeless eggs, and isolated polar lobes were resolved by one-dimensional electrophoresis on SDS gels, as shown in Figures 2A, B, and C. These fluorographs show the incorporation of methionine into two variants of H1 histones as well as into other histones, of which H2A and H2B are not individually resolved.

Densitometric scans of the fluorographs of histones in Figures 2A, B, and C were made, and the area under the scans was determined as a measure of the accumulation of radioactivity into the H4 histone for each region of the egg. Histone H4 was selected as that histone least likely to be contaminated by traces of non-histone peptides that had incorporated the high specific activity ^{35}S-methionine

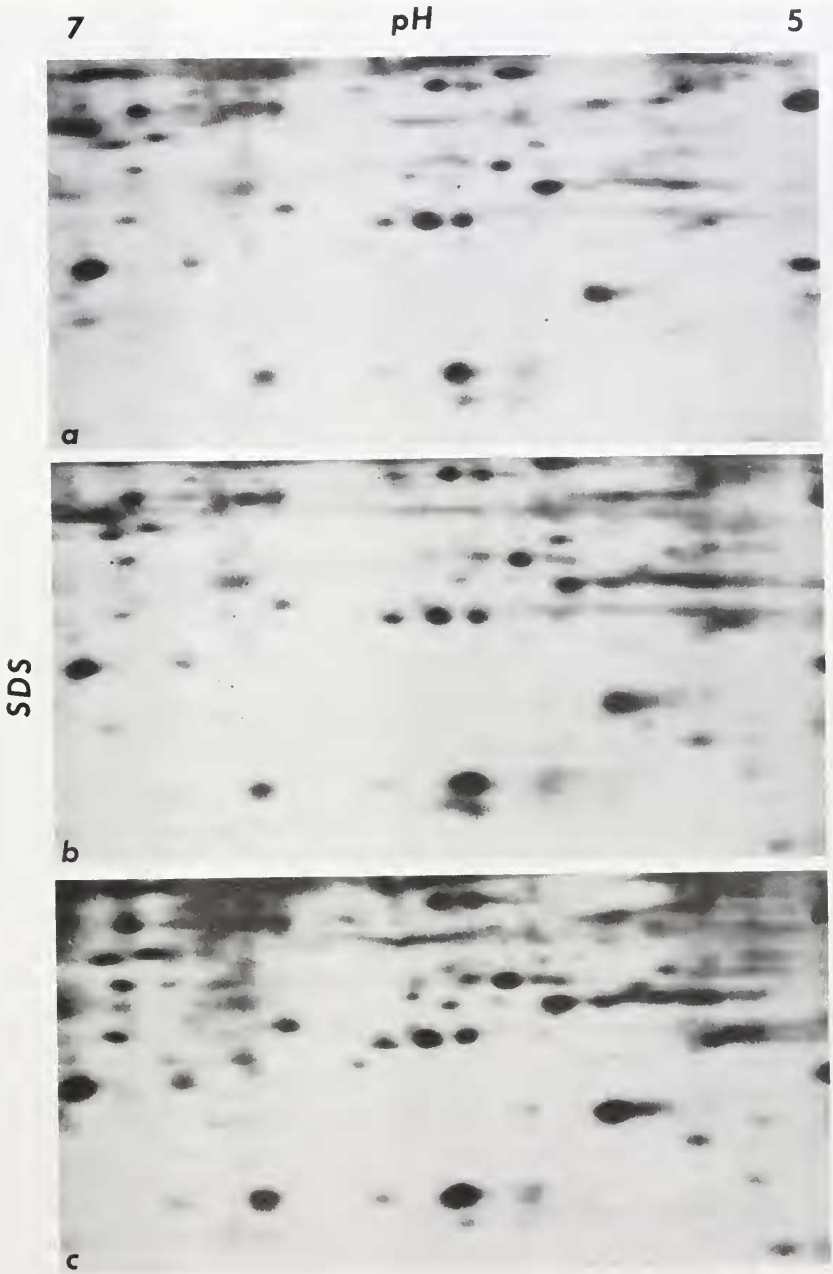

FIGURE 1. Autoradiograph of ^{35}S-methionine *in vivo* labeled polypeptides synthesized during early cleavage by (a) normal embryos, (b) lobeless embryos, and (c) isolated polar lobes.

used in these experiments. These measurements showed that the H4 of the lobeless egg and the isolated polar lobe accumulated 71.2% and 20.7%, respectively, of the amount of methionine incorporated into H4 by the intact egg. This distribution of

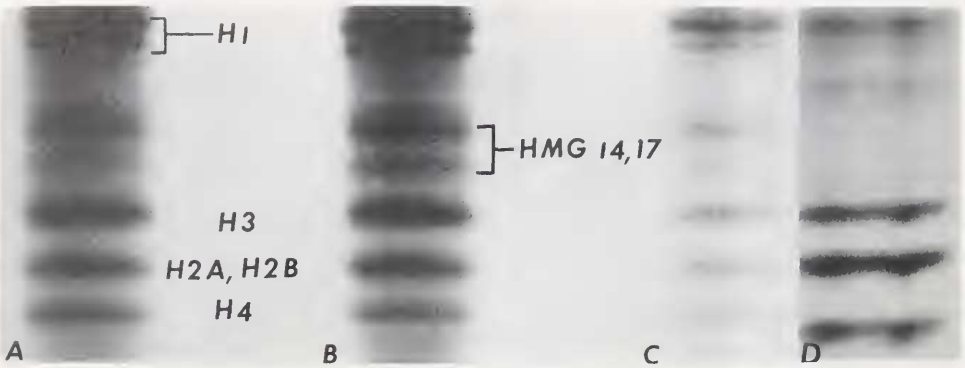

FIGURE 2. Autoradiogrph of *in vivo* labeled histones (H1, H2A, H2B, H3, and H4) and high mobility group peptides 14 and 17 synthesized during early cleavage by (a) normal embryos, (b) lobeless embryos, and (c) isolated polar lobes. In panel (D) are stained histones extracted from isolated nuclei of *Ilyanassa* embryos used, as described in Materials and Methods, to identify the position of histones on the autoradiographs.

label into H4 in these regions of the egg approximates the 84.2% of the volume of non-yolk cytoplasm in the lobeless egg and 16.5% in the polar lobe. Thus, the lobeless egg and the polar lobe probably make histones at the same relative rate, and the histone mRNAs probably are not differentially sequestered into either of these regions of the egg.

Two prominent radioactive bands positioned between the H1 and H3 histones also are resolved in the fluorograph of Figure 2. The location of these bands corresponds to the position of high mobility group peptides 14 and 17 as resolved by SDS acrylamide electrophoresis by Goodwin *et al.* (1979) and Gordon *et al.* (1981). Accordingly, these radioactive bands are probably HMG peptides 14 and 17, and, as shown in these figures, are synthesized not only in the intact egg (Fig. 2A), but also in the lobeless egg (Fig. 2B) and in the isolated polar lobe (Fig. 2C).

DISCUSSION

The nearly complete inhibition of protein synthesis in early-cleavage-stage embryos by cycloheximide demonstrated that mitochondria contribute less than 4% to polypeptide synthesis during cleavage. These results, the observations of Clement and Lehman (1956) that the mitochondria of the *Ilyanassa* egg are concentrated in the animal half of the egg, and the electron micrographs of Pucci-Minafra *et al.* (1969), which also show a paucity of mitochondria in the polar lobe, establish that mitochondria do not contribute significantly to the polypeptide synthesis of the polar lobe or other regions of this egg.

The equivalent rates of protein synthesis measured in different regions of the egg (Table II) could have been an average produced by variable rates of synthesis of different subsets of peptides in the lobeless egg and the polar lobe. However, the similarity in spot size and apparent density of the peptides resolved by two-dimensional electrophoresis (Figs. 1A, B, and C) indicates that all of the detectable proteins made at this stage of development are synthesized at the same relative rate by both the lobeless egg and the isolated polar lobe.

The similar relative rates of protein synthesis in the lobeless egg and the isolated polar lobe indicate that at this stage of development ooplasmic segregation does

not differentially localize into the lobeless egg or the polar lobe amino acids (as inferred from the distribution of ^{35}S-methionine) nor rate-limiting components required for protein synthesis (tRNA, Met-tRNA, 40S ribosomal subunits, initiation factors and so on). Therefore, any qualitative differences in polypeptide synthesis in these regions of the egg would not be caused by differences in the amino acid pool nor by competition among mRNAs for rate-limiting components required for protein synthesis. It is possible that the polar lobe does not contain an equivalent complement of these rate-limiting components, but its rate of protein synthesis is equivalent to that of the egg or lobeless egg because the polar lobe contains a higher concentration of mRNA than the egg or lobeless egg. However, this seems unlikely because of the similar concentrations of total RNA in the polar lobe and the whole egg (Collier, 1960), and because polar lobe removal does not alter the proportion of nascent RNA polyadenylated by lobeless eggs during early cleavage (Collier, 1975).

The fluorographs of peptides made by the egg and its isolates (Fig. 1) show that the lobeless egg and the polar lobe contain, within the limits of the peptides detected, the same set of mRNAs and that these mRNAs are not differentially translated in either the animal or vegetal half of the egg. The similarity of the peptides synthesized by the normal egg (Fig. 1A) and the lobeless egg (Fig. 1B) demonstrates that the polar lobe cytoplasm does not regulate, either quantitatively or qualitatively, the proteins whose synthesis was detected during early cleavage. Obviously, these conclusions apply only to the mRNAs whose translation products are labeled by *in vivo* incubation with ^{35}S-methionine and resolved by two-dimensional electrophoresis. The mRNAs that code for the polypeptides detected by two-dimensional electrophoresis probably belong to the super-abundant and moderately prevalent classes of mRNAs (Davidson and Britten, 1979).

Because the same set of peptides synthesized in the normal egg (Fig. 1A) were also made in the isolated polar lobe (Fig. 1C), the availability of the mRNAs coding for these peptides probably did not depend on concurrent gene transcription. Because the chromosomes of the *Ilyanassa* egg were in a nontranscribing stage, *e.g.* meiotic and mitotic divisions, continuously since before fertilization and the time when the polar lobe was isolated, the mRNAs for these proteins probably were transcribed during oogenesis and stored in the egg cytoplasm. This may apply only to the translation of mRNAs contained in the isolated polar lobe; the maternal transcripts of the egg may have been supplemented by mRNAs transcribed from the nuclear genome of the egg during the 4 h incubation with ^{35}S-methionine, *i.e.* from the two- to the twelve-cell stage. However, experiments with eggs continuously incubated in actinomycin D (Collier and McCarthy, in press) ruled out this interpretation by showing that all of these peptides were translated from maternal transcripts and that these maternal mRNAs were not supplemented by embryogenic transcripts during early cleavage.

The present observations on polypeptide synthesis show that the absence of the polar lobe has no discernible effect on protein synthesis during early cleavage, *i.e.* from the two- to the twelve-cell stage. Accordingly, within the limits of polypeptide detection, the expression of structural genes is not associated with the lobe-dependent determinative events of early cleavage. The absence of an immediate effect is not surprising in the light of Clement's (1962) findings that some of the principal morphogenetic influences of the polar lobe are mediated after the 12-cell stage of development.

The similar rates of synthesis of histones in the egg, lobeless egg, and isolated polar lobe shows that the histone mRNAs are not uniquely localized in the animal

or vegetal half of the *Ilyanassa* egg, and are translated with equal effectiveness in both regions of the egg. Since the mRNAs for the histones and the HMG peptides 14 and 17 are translated in the isolated polar lobe, their presence does not depend on concurrent gene transcription. Because the chromosomes of the *Ilyanassa* egg were, as discussed above, in a non-transcribing stage before isolation of the polar lobe, the mRNAs for these groups of proteins probably were transcribed during oogenesis. It has not been established that synthesis of these basic proteins is insensitive to actinomycin D. Therefore, the maternal transcripts of histone and HMG peptides may have been supplemented by embryogenic transcripts of histone and HMG peptides produced from the two- to the twelve-cell stage, as, for example, has been demonstrated during early sea urchin embryogenesis (Ruderman and Gross, 1974; Easton and Whitely, 1979).

Acknowledgments

I thank Miss Arlene Hughes and Miss Mona Friedman for their excellent technical assistance. This research was supported by grants 11624 and 12276 from the City University of New York PSC-BHE Research Award Program and by grant PCM78-02773 from the National Science Foundation.

LITERATURE CITED

Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1:** 279–285.

Clement, A. C. 1952. Experimental studies on germinal localization in *Ilyanassa*. I. The role of the polar lobe in determination of the cleavage pattern and its influence in later development. *J. Exp. Zool.* **121:** 593–626.

Clement, A. C. 1956. Experimental studies on germinal localization in *Ilyanassa*. II. The development of isolated blastomeres. *J. Exp. Zool.* **132:** 427–446.

Clement, A. C. 1962. Development of *Ilyanassa* following removal of the D macromere at successive cleavage stages. *J. Exp. Zool.* **149:** 193–216.

Clement, A. C., and F. E. Lehman. 1956. Uber das Verteilungsmuster von Mitochondrien un Lipoidtropfen wahrend der Furchung des Eies von *Ilyanassa obsoleta* (Mollusca, Prosobranchia). *Die Naturwiss.* **20:**478–479.

Clement, A. C., and A. Tyler. 1967. Protein-synthesizing activity of the anucleate polar lobe of the mud snail *Ilyanassa obsoleta*. *Science* **158:** 1457–1458.

Collier, J. R. 1957. A study of alanylglycine dipeptidase activity during the development of *Ilyanassa obsoleta*. *Embryologia* **3:** 1–17.

Collier, J. R. 1960. The localization of ribonucleic acid in the egg of *Ilyanassa obsoleta*. *Exp. Cell Res.* **21:** 126–136.

Collier, J. R. 1975. Polyadenylation of nascent RNA during the embryogenesis of *Ilyanassa obsoleta*. *Exp. Cell Res.* **95:** 263–268.

Collier, J. R., and M. E. McCarthy. 1979. Protein synthesis in the polar lobe of the *Ilyanassa* egg. *Amer. Zool.* **19:** 950.

Collier, J. R., and M. E. McCarthy. In press. Regulation of polypeptide synthesis during early embryogenesis of *Ilyanassa obsoleta*. *Differentiation* **19.**

Crampton, H. E. 1896. Experimental studies on gasterpod development. *Wilhelm Roux Arch. Entwicklungsmech.* **3:** 1–19.

Davidson, E. H., and R. J. Britten. 1979. Regulation of gene expression: possible role of repetitive sequences. *Science* **204:** 1052–1059.

Easton, D. P., and A. H. Whiteley. 1979. The relative contribution of newly synthesized and stored messages to H1 histone synthesis in interspecies hybrid echinoid embryos. *Differentiation* **12:** 127–133.

Garrells, J. I. 1979. Two-dimensional gel electrophoresis and computer analysis of proteins synthesized by clonal cell lines. *J. Biol. Chem.* **254:** 7961–7977.

Goodwin, G. H., C. G. P. Mathew, C. A. Wright, C. D. Venko, and E. W. Johns. 1979. Analysis of the high mobility group proteins associated with salt-soluble nucleosomes. *Nucleic Acid Res.* **7:** 1815–1835.

GORDON, J. S., J. BRUNO, AND J. J. LUCAS. 1981. Heterogeneous binding of high mobility group chromosomal proteins to nuclei. *J. Cell Biol.* **88:** 373–379.

LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.

MCCARTHY, M. E., AND J. R. COLLIER. 1980. Regulation of polypeptide synthesis in the *Ilyanassa* embryo. *J. Cell Biol.* **83:** 418.

NEWROCK, K., AND B. BRANDHORST. 1979. Selective translation of cytoplasmic messenger RNA during embryonic development. *J. Cell. Biol.* **83:** 418.

O'FARREL, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250:** 4007–4021.

PUCCI-MINAFRA, S. MINAFRA, AND J. R. COLLIER. 1969. Distribution of ribosomes in the egg of *Ilyanassa obsoleta*. *Exp. Cell Res.* **57:** 167–178.

RUDERMAN, J. V., AND P. R. GROSS. 1974. Histones and histone synthesis in sea urchin development. *Dev. Biol.* **36:** 286–298.

SCHATZ, G., AND T. L. MASON. 1974. The biosynthesis of mitochondria proteins. *Ann. Rev. Biochem.* **43:** 51–88.

WILSON, E. B. 1928. *The cell in development and heredity*. Macmillan Co., New York. 1231 pp.